Chapter

Concepts and Trends for Extraction and Application of Microalgae Carbohydrates

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Abstract

The proposed chapter aims to provide a more in-depth explanation of the composition of carbohydrates in microalgae biomass, focusing on separation methods, chemistry, molecular characterization, as well as their application in several areas. The purpose of this review chapter is to show that biological products from microalgae have potential in health, food, and industry applications (materials and biofuel production). Steps for extraction and purification will be discussed, as well as the relationship between the type of microalgae and its composition, as a way of optimizing protocol selection and product making, without breaking down the cell to begin with (total carbohydrate extraction present in the cell). An overall overview of the current and prospective trends and methodologies for the use of microalgae carbohydrate will be included as starting points to shed light on some of the possible issues that currently do not allow the development and feasibility of microalgae biorefineries.

Keywords: microalgae, carbohydrates, analytical chemistry, biorefinery, trends

1. Introduction

The constant necessity of looking for new alternatives to produce sustainable versions of products has led to the discovery and development of new technique and biological models. Organisms with the potential of producing molecules that can be used for the development of bioproducts in different areas (food, beauty, health, and biodiesel, among others) have led to the discovery, study, and use of new organisms. Algae and microalgae have become potential and promising model organisms to be used for carbohydrate production and use, but besides that, it is also deeply studied due to its lipids, proteins, and photosynthetic activity, making them a prospective source of bioenergy production [1, 2].

The problems associated to the first- and second-generation feedstock for biofuels have seen to become more and more complicated to solve considering the food crisis and complex conversion of lignocellulosic materials [3] researchers, has led to the exploration of a third-generation feedstock, mostly represented by photosynthetic organisms, primarily algae/microalgae [4].

Microalgae are considered to have the potential to produce third-generation biodiesel (due to its capability of fixing carbon dioxide (CO_2), which is eventually converted to biomass and other products), which can also be referred as third-generation feedstock, providing mainly lipids, proteins, and carbohydrates. The utilization of these molecules in a sequential way allows the treatment of biomass in biorefineries, including its use in fermentative production of a range of platform biochemical [5]. Through this process, carbohydrates are used as a fundamental piece for the production of certain products.

Besides the cell wall and reserves of photosynthetic reaction, the carbohydrates can be excreted by the cell. The exopolysaccharides (EPS) are complex carbohydrates produced from some microalgae, which are long chains composed of sugar derivative structures, mucilaginous and with reactive functional groups, such as sulfate, hydroxyl, or carboxylic [6]. The major components of EPS include mainly the polysaccharides and others as proteins, nucleic acid, and lipids [7]. Addition of these molecules is considered to be of extreme importance for enriching the nutritional value of food items [8]. Although these characteristics are beneficial, the extraction of these compounds from microalgae becomes a real challenge. In this context, several treatments can be performed for the disruption of microalgal cells, including chemical modifications and mechanical, thermal, or ultra-sonication processes [9].

Although promising, it is still hard to manage the cost and work that developing new technologies have for investors (in the industry for applied approaches and academia for basic development and standardization), which presents some limitations for the advance of research in this area. On the long run, an implementation of the use of microalgae as a substitute for many of the other crop options still used will have an important impact on the economics, environment, and more sustainable practices.

Due to the vast diversity of species of microalgae, we present an analysis of the current trends and importance and potential of the use of carbohydrates present in

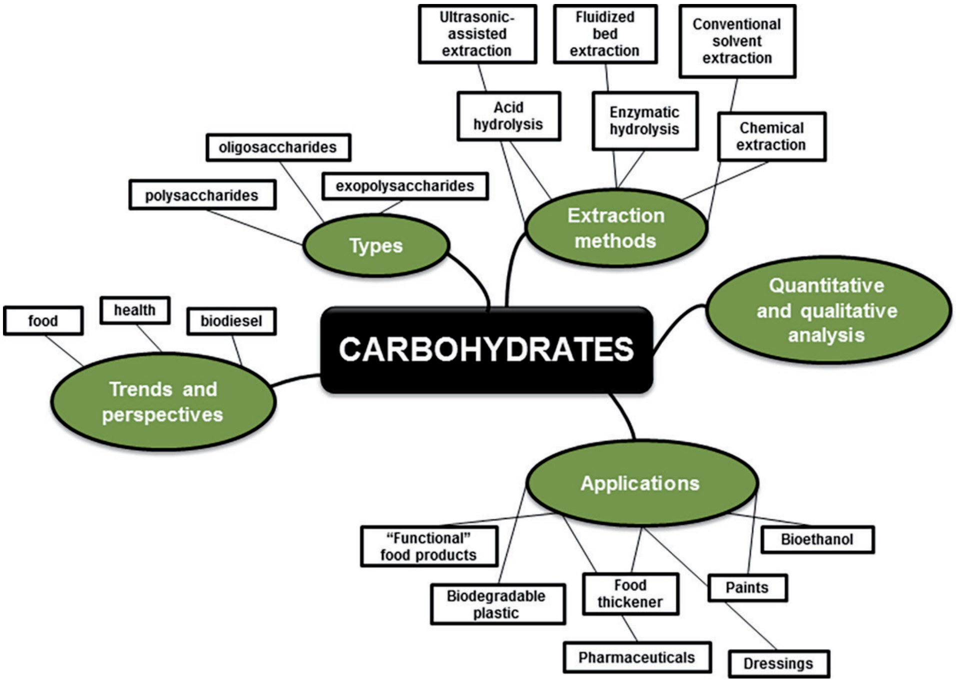

Figure 1.
Diagram of the main topics involved in the harnessing of microalgae carbohydrates.

microalgae. The aim of highlighting these points is to bring awareness and present new alternatives of methods that will allow the use of carbohydrates in microalgae without the breakdown of these carbohydrates, as a way of improving and lowering cost and making the use of these organisms a more feasible way of developing new products and technologies [10]. The main topics that will be discussed in this chapter can be seen in **Figure 1**.

2. Carbohydrate importance and composition

Carbohydrates from microalgae are considered to have a great application in industry, which has led to the development of new techniques and studies but that has exposed new challenges to industry [11, 12]. The diversity of microalgae, the composition, and cell organization are some of the few trials that many scientists are currently facing.

Carbohydrates are poly- or oligosaccharides that can be present in vacuoles and cell walls or that could also be excreted as exopolysaccharides (EPS) [13]. Microalgae come to be an interesting key organism to study due to the high content

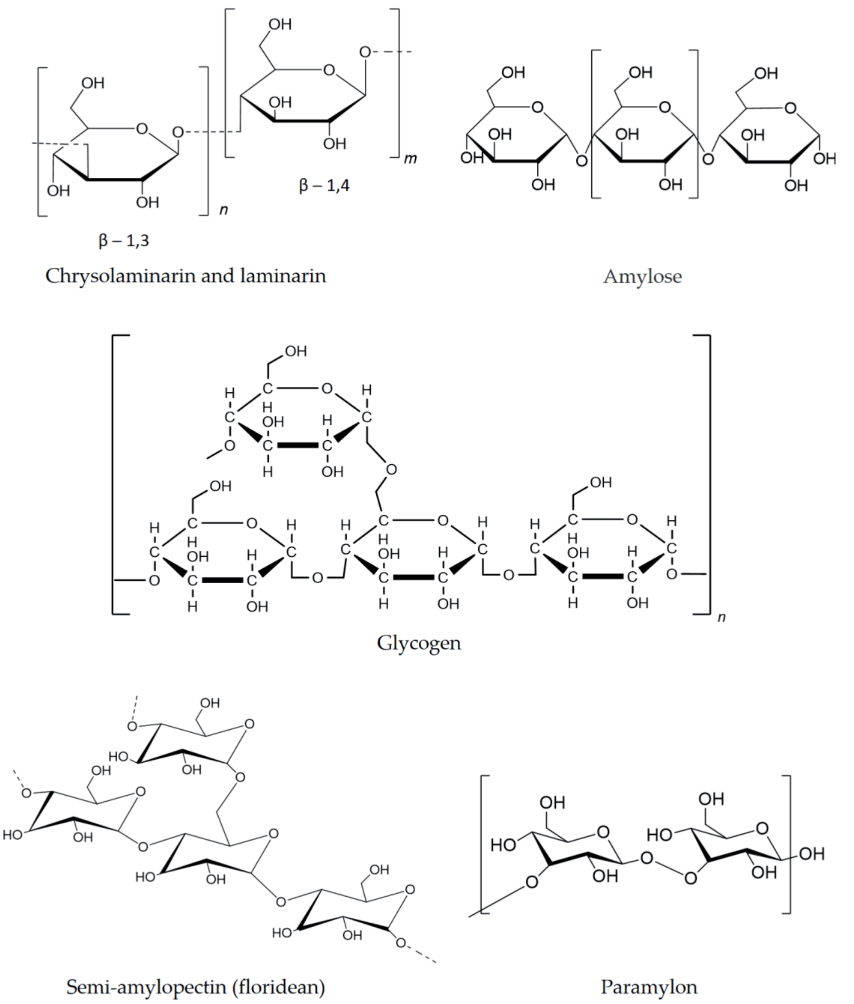

Figure 2.
Glucose polymers found in microalgae.

of carbohydrates that some of them have. Some examples are *Desmodesmus spp*. (41%) from wastewater and landfill leachate treatment [14], *Chlorella stigmatophora* (~55%) [5], or *Chlorella vulgaris* (60% [15] or >52% [16]) cultivated under conditions of nitrogen depletion.

Similarly, indigenous microalgae species have been bioprospected in nitrogen-depleted environments, such as *Desmodesmus sp*. (57%), an unidentified one with more carbohydrate content (70%) [17], and *Arthrospira platensis* that accumulated up to 74% of carbohydrates [18].

Despite their vast potential, using different species imposes a few challenges when trying to establish a consistent methodology for extraction and use. Based on the differences in metabolism that each species presents, a possible and feasible approach is to consider that they all have reserves made out of polymers of glucose (glucans), such as chrysolaminarin (1:11), laminarin (3:1) (β-1,3 and β-1,6 branches), paramylon(β-1,3), glycogen-type, cyanophycean, floridean (semi-amylopectin: α-1,4 and α-1,6 branches), and amylose-type starch (α-1, 4) or both (**Figure 2**). The external covering of cell with polysaccharides could be peptidoglycan matrices, cellulosic wall, and galactose polymer matrix, and others [18–20].

The potential of using microalgae for the development of various products, using carbohydrates as the main source, exposes some of the challenges that using these organisms can have. The main of it is the process of extraction of the diverse saccharides present in each species, but in comparison with plant-derived products, it is actually a much easier path, since they do not present lignification of the cell wall [11, 21]. In the same way, having various species together as a main feedstock source may require adaptations to a proper and more efficient method of extraction.

3. Trends for microalgae use and production, using carbohydrates as the main molecule of interest

Microalgae use for development of new products that could be beneficial to multiple industries has raised many questions in the quest of finding what will be the next technology that will be developed, what products will change our industries, and how this could benefit populations. As we know, the use of microalgae carbohydrates could be a solution to many current limitations that agriculture, pharmacy, nutrition, and other areas are facing while trying to develop better solutions to fulfill the needs of people [22].

One of the main reasons for the development of new technologies and the arise of trends is to be able to satisfy the demands of the public and produce the income that the market will like to have in return for investing in these areas. Unfortunately, it is still a difficult task since there is still a long way to go for developments of products outside of the food industry.

Carbohydrates/starch is considered to be a positive/beneficial step for the chemical development of products, and when produced in high quantities, its use for fermentation is considered a better option. To date, the most common way to induce the production of specific molecules (in this case, carbohydrates and starch) is by creating stress conditions in the environment where the alga is growing, mainly by altering nutrient concentrations or by changing light, temperature, and other parameters [23, 24].

Photosynthetic electron transport approach seems to be the most researched area [25] since genetic modification of algae becomes challenging due to the great variety of species. Cyanobacteria are being used more for genetic engineering, but no complete success has come out it yet. Besides the interest cyanobacteria, some algae species have been explored as potential species for biofuel production through modification of metabolic pathways that could increase the production of carbohydrates, lipids, and other compounds, but the progress in this area has seen to be slow, which although discouraging is still considered one of the main focus for the future of biotechnologies in microalgae [26].

Microbiome studies of microalgae populations seem to focus more on the ecology aspects and the importance of their use in multiple biological processes. Toxicity of waters seems to attract the attention of most research groups. Its importance lies in not only the understanding of structure communities and safety of fresh water resources (which is the main source of drinking water for the world) but also the discovery of new species that may be hard to culture through regular isolation practices.

Similarly, human microbiome studies seem to be interested in the potential use of carbohydrates (including exopolysaccharides) derived from microalgae, due to its prebiotic potential, improving the health of adults and infants that cannot consume breast milk in their early stages, strengthening their immune responses [12, 27].

4. Carbohydrate extraction and analysis

Some microalgae species are capable of accumulating a large amount of intracellular starch and have structural polysaccharides in their cell walls [28]. However, the development of standard analytical procedures for the characterization of microalgae biomass has been difficult because of the existence of several microalgae species with different cellular structures and chemical compositions [28]. Even a specific microalgae species may present variability in their chemical composition because they can strongly be affected by factors of cultivation conditions such as temperature, salinity, and nutrient availability [9]. Thereby, sample preparation for the determination of carbohydrates in microalgae is very complex.

The analytical method applied depends on the intended usage of the algal carbohydrates. Some require the qualitative or quantitative composition of the monosaccharides and others the total carbohydrate analysis [29].

In algal biomass, the carbohydrate profile analysis requires preliminary acid hydrolysis. Acid hydrolysis allows depolymerizing the intracellular starch and structural polysaccharides into their monomers, which are then further quantified [28]. The optimal hydrolysis conditions (acid, temperature, and time) should ensure complete hydrolysis of the polysaccharides and at the same time avoid excessive degradation of monosaccharides [29].

In this context, the selective cleaving of algal polysaccharides by enzymatic hydrolysis is another promising approach once the formation of degradation products like furfural or hydroxymethylfurfural is avoided [29]. However, for some microalgae species, the composition of the cell wall is complicated and unknown; also, some enzymes are very expensive [30]. The cell wall present in the microalgae limits extraction yields of high-value products or results in a low bioavailability of intracellular components [9, 30].

The methodology widely employed in acid hydrolysis is the procedure published by the National Renewable Energy Laboratory (NREL) based on two-step hydrolysis using H_2SO_4 [31, 32]. Despite being a reliable method, it is a multistep procedure, increasing the chance of experimental errors, and also presents a high time of sample preparation. Northcote et al. [33] proposed another method based on one-step acid hydrolysis with dilute H_2SO_4 and use smaller biomass samples.

Other methods use chemical extraction method like alkaline pretreatment [34] and physical method, such as hot-water treatment, microwave-assisted extraction, and ultrasonic-assisted extraction [30, 35]. The choice of extraction method is that the pretreatment is effective qualitatively and quantitatively, and the technology is simple to operate and economical for scale-up [30].

Zhao's research team [30] investigated three methods of conventional solvent extraction (CSE), fluidized bed extraction (FBE), and ultrasonic-assisted extraction (UAE) to obtain an effective extraction method of carbohydrates/glucose. The CSE employed lyophilized microalgae extracted with distilled water and agitation in a vortex. For FBE, the *Chlorella* sp. culture was harvested and washed with distilled water and then diluted using distilled water and added into a fluidized reactor with air aeration. To UAE, the algal cells were harvested and washed with distilled water, diluted, and taken to the ultrasonic processor. The ultrasonic-assisted extraction was more effective than the other methods.

Information in the literature related to the amount of cell wall microalgae polysaccharides is scarce. Usually, the quantification of polysaccharides in microalgal is made by analyzing the total carbohydrate, thus including storage polysaccharides (SPS) and cell wall-related polysaccharides, which exhibit different functions in the microalgal cell [9].

According to Bernaerts et al. [9], the insight into the composition of cell wall-related polysaccharides, such as the monosaccharide profile or the degree of sulfation, is not only desired in terms of process optimization but also as a potential for several biotechnological. Thereby, the authors investigated to apply a universal procedure for extraction of the total cell wall-related polysaccharides, including cell wall polysaccharides (CWPS) and extracellular polymeric substances (EPMS), of 10 commercially available microalgae species followed by a characterization of the monosaccharide profile, uronic acid content, and sulfate [9]. Initially, the procedure consisted of dry biomass suspended in saline solution incubated for 16 h at 25°C, followed by a two-step centrifugation. Afterward, the supernatant was submitted for extraction of EPMS and the residual biomass (pellet) for extraction of CWPS. Ethanol was added to the supernatant precipitating EPMS; the solution was vacuum filtered, and the insoluble residue was dialyzed against demineralized water for 48 h, and finally, the extracts were lyophilized.

In the extraction of CWPS, the pellets were suspended in MOPS buffer, and the cells were disrupted using UHPH after cold ethanol was added to the suspensions and for pellet recovery. Lipids were removed by addition of hexane/isopropanol to the pellet, mixed and centrifuged to remove the upper solvent layer. Afterward, SPS and protein were enzymatically removed using endo-β-1,3-glucanase or a combination of α-amylase/amyloglucosidase and *Subtilisin* A protease, respectively. The mixtures were incubated and after addition of cold ethanol they were centrifuged. The pellet was finally washed in acetone, vacuum filtered, and dried overnight at 40°C, and this residue was considered as CWPS. Monosaccharide and uronic acid composition of CWPS and EPMS were hydrolyzed according to De Ruiter et al. [36] using methanolysis combined with trifluoroacetic acid (TFA) hydrolysis.

After sample preparation, carbohydrate analysis is a very complex field. Usually, after microalgal acid hydrolysis, the total carbohydrate content of the hydrolysate can be determined using colorimetric procedures like the phenol-sulfuric acid [37–39] or anthrone-based [40–42]. These methods are available, giving excellent and robust results with low effort in a very short time. Nevertheless, detailed information about the monosaccharide composition cannot be generated [29]. Qualitative investigations can be performed using TLC methods with silica-based separation materials making the separation of most monosaccharides possible. However, quantification with the TLC methods is not possible [29], and for quantification of monosaccharides, analytical methods such as high performance liquid chromatography (HPLC) are often used. The HPLC equipped with a refractive index detector (RID) [32] and HPLC combined with pulsed amperometric detection (HPAEC-PAD) [9, 28] and liquid chromatography mass spectrometer (LC–MS) [29] were related.

The trends for carbohydrate analysis are the exploration of methods that study both cell wall polysaccharides and extracellular polymeric substances. The developed methods always aim to use small amounts of sample, reagents, and shorter analysis. Liquid chromatography has been highlighted in the carbohydrate determination since it presented a good separation and quantification of these compounds. Although it is a more expensive analytical technique, it provides data on the composition of individual monomeric sugars that make it of interest for this type of analysis.

5. Applications

Microalgae have several types of polysaccharides in their composition, such as phycocolloids, agar, alginate, carrageenan, fucoidan, ulvana, and cellulose, among others. phycocolloids can be formed by different monomers such as glucose, galactose, mannuronic acid, guluronic acid, mannitol, and laminarin. These carbohydrates can be inserted into functional beverages and food products such as functional bread, ready to serve soups, functional snack foods and a variety of sauces, creams, bakery products, and additional food products [43, 44].

Due to the high carbohydrate content, poultry and aquaculture feed is one of the main study targets for the use of microalgae biomass. In 2007, around 30% of the world's current algae production was sold for animal feed application [8]. Microalgae are also a suitable alternative for growing fish, larvae, and zooplankton. *Chlorella* is one of the main examples of microalgae that can play a key role in food and feed due to the properties of its biomass, which can simultaneously provide high concentrations of carbohydrates, vitamins, and proteins [45]. Besides *Chlorella*, other species used in aquaculture can be highlighted: *Tetraselmis*, *Isochrysis*, *Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Nannochloropsis*, *Skeletonema*, and *Thalassiosira*. *Spirulina* and *Chlorella* microalgae can be applied in the feeding of cats, dogs, aquarium fish, ornamental birds, horses, birds, cows, and breeding bulls. The most common genera of larval microalgae include *Chaetoceros*, *Thalassiosira*, *Tetraselmis*, *Isochrysis*, and *Nannochloropsis* [46].

1,3-β-glucan is an important carbohydrate present in microalgae composition due to its applications in the food industry as a thickener, and health applications, especially in the protection against infections and also to inhibit cancer cell growth in vivo [44, 47]. According to [48], the global β-glucan market was valued at USD 307.8 million in 2016, and it is predicted that in 2022, the global carbohydrate market could reach up to USD 476.5 million, which indicates the huge potential for development in many different types of applications.

According to Koller et al. [49], sulfated polysaccharides produced by microalgae can be applied in therapies against bacterial infections. Carrageenan polysaccharide, also known as food additive E407, can be used in pharmaceutical applications. Marine carbohydrates have been widely used in the cosmetics industries due to their chemical and physical properties. Brown algal fucoidans/alginates, green algal ulcers, and red algal carrageenans/agar are used as gelling, thickening, and stabilizing agents. In addition, marine carbohydrates have potential skin benefits, and biological activities are linked to their structure as determined by molecular weights or the presence of sulfate groups and other sugars [50].

Red algae, such as *Chondrus* sp., *Gigartina* sp. *Eucheuma* sp., *Hypnea* sp., and *Furcellaran* sp., are widely used for the production of carrageenan. This compound can be used in food and pharmaceutical industries for applications in fruit gel, fruit juices, sweets, and jellies, among others. Another carbohydrate group molecule is fucoidan, which is associated with brown algal cell wall components (*Phaeophyceae*). Among the bioactivities derived from this molecule, the anticoagulant, antitumor, antivirus, and antioxidant properties stand out, making it attractive for pharmaceutical applications [51].

Besides these applications, the remaining biomass of microalgae presents carbohydrate-rich molecules, which have been widely used in the production of bioplastics, agar, sugars, and other high-added value chemicals. However, despite being a growing area, the biorefinery stage must be studied in order to extend its applicability on an industrial scale [51]. According to Mihranyan [52], the rheological behavior of cellulose found in *Cladophora* algae is similar to micro fibrillated cellulose. Because this cellulose is very robust and not susceptible to chemical reactions, the properties of cellulose found in these algae provide excellent rheological properties making this material interesting in food, pharmaceuticals, paints, dressings, and biodegradable plastic applications.

The high carbohydrate content and low-ash values make microalgae more suitable for conversion to biofuels [43]. The production of bioethanol from microalgae gained importance due to their high biomass productivity, diversity, variable chemical composition, and high photosynthetic rates of these organisms [53]. Due to the large amount of carbohydrates/polysaccharides and cellulose walls, these microorganisms become favorable for the production of this biofuel [54, 55]. In many countries, ethanol is produced on a large scale from crops containing sugars and starches in its composition through fermentation. The biomass is ground, and the starch is converted into sugars by different methods. Polysaccharide starch is also accessible as a storage material for various algal species and can be anaerobically converted into bioethanol [49].

6. Conclusions

Microalgae biomass conversion technologies involve carbohydrates as the main source in the production of biofuels and other compounds of high commercial value. Changes in metabolic pathways aiming at increased carbohydrate production are seen as a potential for enhancing microalgae biotechnology. Extraction methods and trends in analytical methodologies focus on microalgae cell wall polysaccharides and the polymers excreted by these microorganisms. The high carbohydrate content makes microalgae excellent candidates for the production of numerous biocomposites, especially beta-glucan, which is on the international market, indicating its strong potential for its use in different biotechnological applications.

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